Exercise Training Increases Glycogen Synthase Activity and GLUT4 Expression But Not Insulin Signaling in Overweight Nondiabetic and Type 2 Diabetic Subjects

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Exercise training improves insulin sensitivity in subjects with and without type 2 diabetes. However, the mechanism by which this occurs is unclear. The present study was undertaken to determine how improved insulin signaling, GLUT4 expression, and glycogen synthase activity contribute to this improvement. Euglycemic clamps with indirect calorimetry and muscle biopsies were performed before and after 8 weeks of exercise training in 16 insulin-resistant nondiabetic subjects and 6 type 2 diabetic patients. Training increased peak aerobic capacity (Vo_{2peak}) in both nondiabetic (from 34 ± 2 to 39 ± 2 mL O_2/kg fat-free mass [FFM]/min, 14% ± 2%, $P < .001$) and diabetic (from 26 ± 3 to 34 ± 3 mL O_2/kg FFM/min, 32% ± 4%) subjects. Training also increased insulin-stimulated glucose disposal in nondiabetic (from 6.2 ± 0.5 to 7.1 ± 0.7 mg/kg FFM/min) and diabetic subjects (from 4.3 ± 0.6 to 5.5 ± 0.6 mg/kg FFM/min). Total glycogen synthase activity was increased by 46% ± 17% and 45% ± 12% in nondiabetic and diabetic subjects, respectively, in response to training ($P < .01$ *v* before training). Moreover, after training, glycogen synthase fractional velocity was correlated with insulin-stimulated glucose storage ($r = 0.53$, $P < .05$) and the training-induced improvement in glucose disposal was accounted for primarily by increased insulin-stimulated glucose storage. Training also increased GLUT4 protein by 38% ± 8% and 22% ± 10% in nondiabetic and diabetic subjects, respectively ($P < .05$ *v.* before training). Akt protein expression, which was decreased by 29% ± 3% ($P < .05$) in the diabetic subjects before training (compared to the nondiabetics), increased significantly in both groups ($P < .001$). In contrast, exercise training did not enhance the ability of insulin to stimulate insulin receptor substrate-1 (IRS-1)–associated phosphatidylinositol 3 (PI 3)-kinase activity. The present data are consistent with a working model whereby 8 weeks of exercise training increases insulin-stimulated glucose disposal primarily by increasing GLUT4 protein expression without enhancing insulin-stimulated PI 3-kinase signaling, and that once the glucose enters the myocyte, increased glycogen synthase activity preferentially shunts it into glycogen synthesis.

SKELETAL MUSCLE is the primary site of glucose disposal under insulin-stimulated conditions. In response to insulin binding, insulin receptors on skeletal muscle cells undergo autophosphorylation on tyrosine residues, leading to activation of the receptor tyrosine kinase and subsequent tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1).[1,2] IRS-1 in turn associates with the regulatory subunit of phospatidylinositol 3 (PI 3)-kinase, activating the P110 catalytic subunit,[2,3] which is necessary for mediating insulin's metabolic effects, including GLUT4 translocation, glucose disposal, and increasing the activity of glycogen synthase and hexokinase.[4-6] While the signaling cascade up to this point is well accepted, downstream mediator(s) are less well defined. Akt, a serine/threonine kinase, has recently come to the forefront as a potential downstream mediator of insulin-stimulated PI 3-kinase activity. Of the 3 Akt isoforms, Akt1, and to a lesser extent Akt2, have been shown to be activated in response to insulin in skeletal muscle.[7]

Insulin resistance in skeletal muscle is characterized by decreased insulin-stimulated glucose disposal, as well as decreased hexokinase and glycogen synthase activity.[8-10] On a molecular level, we and others have found insulin's activation of the IRS-1 PI 3-kinase signaling pathway to be reduced in skeletal muscle of rodents and humans with insulin resistance and type 2 diabetes.[11-13] Akt activation in response to insulin is decreased in Zucker Fatty rats, a rodent model of insulin resistance.[14,15] Studies involving type 2 diabetic subjects report conflicting results, with Akt activation reported to be either normal[16,17] or reduced.[18,19]

There is substantial evidence that exercise training improves insulin sensitivity.[20,21] Whether this is due in part or entirely to a reversal of the impairment in insulin signaling is unclear. Exercise training increases mRNA expression of insulin receptor, IRS-1, mitogen-activated protein (MAP) kinase (ERK-1), PI 3-kinase, GLUT4,[22] and glycogen synthase[23]; IRS-1[24] and GLUT4 protein expression,[20,25] as well as glycogen synthase[23] and hexokinase enzyme activity.[26] With regard to insulin stimulation of the PI 3-kinase signaling pathway, studies have shown PI 3-kinase activity to be increased after exercise training in humans and rodents.[21,27] In addition to enhanced PI 3-kinase activity, Luciano et al observed greater Akt serine phosphorylation in response to insulin in male Wistar rats after 6 weeks of training.[21] These results support the idea that improved insulin sensitivity is associated with adaptations in insulin signaling. However, these studies were performed in healthy, insulin-sensitive subjects or rodents.

To date only 2 studies involving insulin-resistant rodents (obese Zucker rats)[14,15] and 1 study involving insulin-resistant

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Table 1. Subject Characteristics Before and After Training

	Controls (n = 16)		Diabetics (n = 6)	
	Before	After	Before	After
Age (yr)	36 ± 2		45 ± 4*	
BMI (kg/m^2)	28.0 ± 0.5	28.1 ± 0.9	28.2 ± 0.5	28.9 ± 0.4
HBA_{1c} (%)	4.8 ± 0.1	4.7 ± 0.1	8.1 ± 1.1†	6.6 ± 0.6†
Vo_{2peak} (mL/kg FFM · min)	34.1 ± 2.1	38.6 ± 2.2‡	25.6 ± 2.4*	33.4 ± 2.7‡
Maximal heart rate (bpm)	167 ± 3	169 ± 3	143 ± 2*	160 ± 4‡
Fasting glucose (mg/dL)	91 ± 2	95 ± 2	133 ± 18*	130 ± 21*
Fasting insulin (μU/mL)	5.9 ± 0.9	5.3 ± 1.1	13.7 ± 4.1†	10.8 ± 3.0†
Ethnicity	7C/6H/ 2AA/1A		1C/5H	

NOTE. Data are given as means ± SEM.

Abbreviations: H, Hispanic; C, Caucasian; A, Asian; AA, African American.

*$P < .05$ *v* controls.

†$P < .01$ *v* controls.

‡$P < .001$ *v* before training.

humans[28] have been published with regard to investigating the effects of training on the insulin signaling pathway. Seven weeks of exercise training did not change either the expression of insulin signaling molecules nor insulin action on insulin receptor and IRS-1 tyrosine phosphorylation, IRS-1–associated PI3-kinase activity, or Akt serine phosphorylation in obese Zucker rats, in spite of an improvement in insulin-stimulated glucose uptake.[14] In contrast, Hevener et al observed an improvement in insulin receptor and IRS-1 tyrosine phosphorylation after 12 weeks of exercise training in association with improved insulin sensitivity in obese Zucker rats.[15] However, they did not see an effect on either PI 3-kinase or Akt activity.[15] The only study thus far specifically investigating insulin-resistant humans was performed in moderately obese (body mass index [BMI], 30) middle-aged men (50 to 70 years of age). After 7 days of exercise training, the glucose infusion rate during a euglycemic, hyperinsulinemic clamp was increased, indicating enhanced insulin action. This was, however, not associated with any change in insulin-stimulated PI 3-kinase activity or Akt serine phosphorylation.[28] Given that the evidence regarding the mechanism of exercise-enhanced insulin action is limited and inconsistent, the present study was undertaken to assess the relative contributions of improvements in insulin receptor signaling, GLUT4 protein expression, and glycogen synthase activity to an increase in insulin sensitivity induced by 8 weeks of exercise training.

MATERIALS AND METHODS

Subjects

Sixteen nondiabetic (6 males/10 females) and 6 type 2 diabetic (4 males/2 females) subjects participated in the study (Table 1). Each subject underwent a complete history and physical examination, including a 75-g oral glucose tolerance test (OGTT) to determine the presence or absence of diabetes using established American Diabetes Association criteria. Nondiabetic subjects did not have a family history of type 2 diabetes and had normal glucose tolerance. Other than having diabetes, diabetic subjects were in good health. Three of the 6 diabetic subjects were taking glyburide, which was withdrawn 3 days prior to clinical studies. The remaining 3 diabetics were treated with diet alone. No subject was taking any other medication known to affect glucose metabolism. No subject participated in a regular exercise program at the time of entry into the study. A normal resting electrocardiogram reading was a prerequisite for participation. Subjects were instructed to consume a diet containing at least 200 g of carbohydrate per day for the 3 days preceding clinical studies and to not exercise on the day before the studies. Body fat percentages were determined using bioimpedance.[29] The study protocol was approved by the Institutional Review Board of University of Texas Health Science Center at San Antonio, and all subjects gave written informed consent.

Study Design

Upon acceptance into the study, subjects underwent determination of their peak aerobic capacity (Vo_{2peak}) and a euglycemic, hyperinsulinemic clamp with muscle biopsies of the vastus lateralis muscle. At least 1 week after the euglycemic, hyperinsulinemic clamp, the subjects began an 8-week aerobic exercise training program. All exercise sessions were supervised by one of the authors. Subjects initially exercised at 60% of their Vo_{2peak} for 20 minutes on a stationary cycle ergometer 3 times per week. Over the course of the 8 weeks, exercise intensity, duration, and frequency were progressively increased to 70% of Vo_{2peak}, for 45 minutes, 4 times per week. Heart rate was used as an indicator of exercise intensity, with subjects exercising at a heart rate corresponding to the appropriate training Vo_{2peak}. At the end of the 8 weeks of training, determination of Vo_{2peak} and the euglycemic, hyperinsulinemic clamp with muscle biopsies were repeated. The euglycemic, hyperinsulinemic clamp at the end of the 8 weeks was done 24 hours after the last exercise bout to allow for any acute exercise effects to subside.

Peak Aerobic Capacity

None of the subjects had participated in a regular exercise program for at least 1 year prior to the study. Vo_{2peak} was determined using an incremental cycle ergometer protocol, with the subject riding until voluntary exhaustion. Criteria for test completion were a respiratory exchange ratio (RER) greater than 1.1 and no further increase in oxygen uptake and/or heart rate. Three subjects (2 controls, 1 diabetic) did not reach a RER greater than 1.1 (average RER = 1.03 ± 0.01). For these subjects, Vo_2 was plotted against RER, and the predicted Vo_2max was taken as the Vo_2 corresponding to an RER of 1.1 (extrapolated from available data). Maximum predicted heart rate was calculated as 220 − age.

Euglycemic, Hyperinsulinemic Clamp

At least 1 week after the Vo_{2peak} test, subjects reported to the General Clinical Research Center (GCRC) at 8 AM after consuming nothing but water since the prior evening to undergo a hyperinsulinemic, euglycemic clamp (Fig 1). An antecubital vein was cannulated for the infusion of glucose, 20% dextrose, and insulin (Humulin; Eli Lilly, Indianapolis, IN). A hand vein was cannulated in a retrograde fashion and the hand was placed in a heated box (60°C) for sampling of arterialized blood. To ensure isotopic equilibrium, a primed (25 μCi × fasting plasma glucose/90), continuous (0.25 μCi/min) infusion of 3-^{3}H-glucose was started 2 hours (nondiabetic subjects) or 3 hours (diabetic subjects) before the start of insulin infusion. Sixty minutes before the start of insulin infusion a percutaneous muscle biopsy of the vastus lateralis muscle was obtained with a Bergstrom cannula under local anesthesia.[30] Muscle biopsy specimens were immediately blotted free of blood, frozen, and stored in liquid nitrogen until used. Arteri-

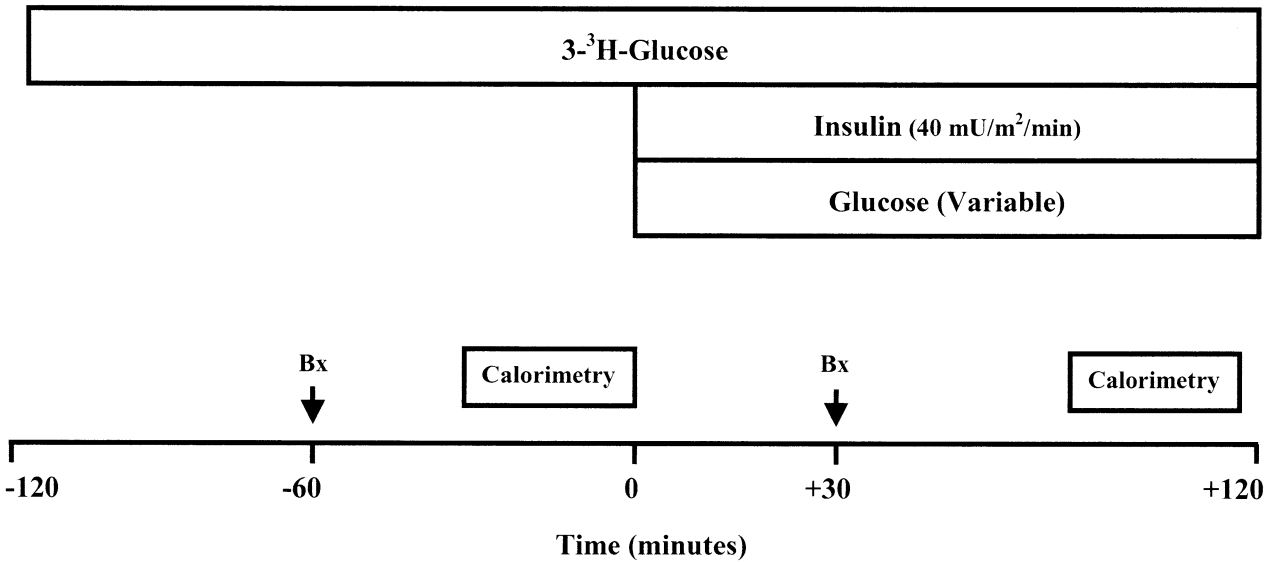

Fig 1. Study design for euglycemic, hyperinsulinemic clamp. Sixteen nondiabetic control and 6 type 2 diabetic subjects underwent a euglycemic, hyperinsulinemic clamp before and after 8 weeks of aerobic exercise training. Tritiated glucose was infused throughout a basal period of 120 minutes for control or 180 minutes for diabetic subjects and during the 120-minute insulin infusion (40 mU/m^2/min). Plasma glucose levels were maintained at euglycemia (90 to 100 mg/dL) by a variable glucose infusion. Muscle biopsies (Bx) of the vastus lateralis muscle were performed basally (−60 minutes) and during the insulin infusion (+30 minutes). Indirect calorimetry was measured for 30 minutes at the end of the basal period (time −30 to 0 minutes) and insulin infusion (time 90 to 120 minutes).

alized blood was sampled for measuring plasma glucose, insulin, and 3-^{3}H-glucose specific activity. Blood samples were obtained at baseline and every 10 minutes during the last 30 minutes of the isotopic equilibration period. Continuous indirect calorimetry was performed with a ventilated hood system (DeltaTrac, Sensor Medics, Anaheim, CA) during the last 30 minutes of the tracer equilibration (basal) and insulin clamp periods for the measurement of carbohydrate and lipid oxidation rates. After completion of the equilibration period, a primed, continuous infusion of insulin was started at a rate of 40 mU/m^2/min for 120 minutes. Plasma glucose was measured every 5 minutes throughout the study with a glucose oxidase analyzer (Beckman Instruments, Fullerton, CA) and maintained at euglycemia (90 to 100 mg/dL) using a variable infusion of 20% dextrose. After 30 minutes of insulin infusion, a second muscle biopsy was obtained from the opposite vastus lateralis muscle.

Materials

Polyclonal anti c-terminal IRS-1 and polyclonal anti-phospho Akt (Ser 473) antibodies were purchased from Upstate Biotechnology (Lake Placid, NY). A polyclonal anti-Akt antibody was purchased from Cell Signaling (Beverly, MA). A polyclonal anti-GLUT4 antibody was purchased from Santa Cruz (Santa Cruz, CA). Platelet-derived growth factor (PDGF)-stimulated NIH3T3 L1 cell lysate served as a positive control for phospho-Akt (Ser 473) immunoblotting and was obtained from Upstate Biotechnology. Rat liver homogenate served as a standard control for the PI3-kinase assay. Goat anti-rabbit and rabbit anti-sheep antibodies coupled to horse radish peroxidase (Piscataway, NJ) were used as secondary antibodies. Protein A was purchased from Sigma Chemical Co (St Louis, MO). [γ-^{32}P] adenosine triphosphate (ATP) was purchased from NEN Life Science Products (Boston, MA). Phosphatidylinositol was purchased from Sigma Chemical (St Louis, MO).

Muscle Processing

Muscle samples were weighed while still frozen and were homogenized in ice-cold lysis buffer (1:10, wt/vol) containing 50 mmol/L HEPES (pH 7.6), 150 mmol/L NaCl, 20 mmol/L sodium pyrophosphate, 20 mmol/L β-glycerophosphate, 10 mmol/L sodium fluoride, 2 mmol/L sodium orthovanadate (Na_3VO_4), 2 mmol/L EDTA (pH 8.0), 1% Nonidet P-40, 10% glycerol, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 10 μg/mL leupeptin, 10 μg/mL aprotinin. A Polytron homogenizer (Brinkman Instruments, Westbury, NY) set on maximum speed for 30 seconds was used for homogenization. Homogenates were incubated on ice for 20 minutes and then centrifuged at 15,000 $\times$ *g* for 20 minutes at 4°C. Cell debris was removed by centrifugation and protein concentrations of crude extracts were estimated by the Lowry method.[31] Supernatants were stored at −80°C until used.

Sodium Dodecyl Sulfate Polyacrylamide Electrophoresis and Immunoblotting

For phospho-Akt (Ser473) and GLUT4, equal amounts of protein were resolved on 7.5% (Akt) or 10% (GLUT4) sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to nitrocellulose membranes. After blocking, the membranes were incubated with antibodies and protein bands were visualized using an enhanced chemiluminescence (ECL) detection system according to the manufacturer's protocol (Amersham). Images were digitized by scanning and band intensity was quantified using Image Tool Software (The University of Texas Health Science Center at San Antonio, San Antonio, TX). For determining Akt expression, the phospho-Akt (Ser473) immunoblot was stripped using a buffer containing 0.7% β-mercaptoethanol, 7 mmol/L SDS, and 6 mmol/L Tris HCl (pH 6.7), for 20 minutes, washed with Tris-buffered saline (TBS) 3 times for 10 minutes each, blocked with TBS-Tween containing 5% milk, and reprobed with anti-Akt antibody overnight. The detection procedures were the same as described above.

PI 3-Kinase Activity

Muscle protein (250 μg) was immunoprecipitated with anti–IRS-1 antibody and PI 3-kinase activity was determined by determining the

Table 2. Lipid Profile Before and After Training

	Control		Diabetic	
	Before	After	Before	After
Total cholesterol	169 ± 8	168 ± 8	204 ± 16*	210 ± 16†
HDL	52 ± 3	53 ± 4	36 ± 2†	36 ± 2†
LDL	95 ± 11	94 ± 6	128 ± 26	121 ± 15*
Triglycerides	107 ± 15	109 ± 16	277 ± 27†	262 ± 52†

NOTE. Data are given as means ± SEM.

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

*$P < .05$ *v* controls.

†$P < .01$ *v* controls.

incorporation of ^{32}P-ATP into ^{32}P-phosphotidylinositol phosphate, as previously described.[14]

Glycogen Synthase Activity

Glycogen synthase activities were assayed using 0.1 ($GS_{0.1}$) and 10 (GS_{10}) mmol/L glucose 6-phosphate, as previously described.[50] Glycogen synthase fractional velocity (GS_{FV}) was calculated as the ratio of $GS_{0.1}$/ GS_{10}. Changes in GS_{FV} are indicative of insulin's effects.

Laboratory Analyses

Plasma insulin concentration was determined by radioimmunoassay (Diagnostic Product, Los Angeles, CA). Plasma tritiated glucose specific activity was determined on barium hydroxide/zinc sulfate-precipitated plasma samples.

Calculations

Glucose disposal rates were calculated using steady-state equations, or where appropriate for non–steady-state conditions, Steele's equation.[31] Glucose and fat oxidation rates were calculated from V_{O_2} and V_{CO_2} data by the equations of Frayn.[33]

Statistical Analysis

All data are expressed as mean ± SEM. Statistical differences among groups were determined using 2-way repeated-measures analysis of variance and Fisher's post-hoc tests, using StatView 4.0 software (SAS Institute, Cary, NC). Correlation analysis was performed by the Pearson product-moment method. For all analyses, $P < .05$ was considered to be statistically significant.

RESULTS

Subjects

Characteristics of the subjects before and after training are shown in Table 1. BMI was similar between the 2 groups and remained constant over the course of the 8 weeks of exercise training. Before training, diabetics had significantly greater fasting plasma glucose, insulin, and hemoglobin A_{1c} (HbA_{1c}) levels than nondiabetic controls. The diabetic subjects also had lower aerobic capacity ($V_{O_{2peak}}$) and lower maximal heart rate before training. HbA_{1c} levels were not affected by training in the control subjects, but there was a trend toward a decrease in HbA_{1c} levels in the diabetics ($P = .06$). The training protocol increased $V_{O_{2peak}}$ by 14% ± 2% for controls and 32% ± 4% for diabetics ($P < .001$), with the percent increase being significantly greater in the diabetic subjects compared to the control subjects ($P < .001$). The maximal heart rate achieved by the diabetics was also significantly increased in response to training (143 ± 2 *v* 161 ± 4 beats per minute, $P < .001$). Fasting lipid profiles before and after training are given in Table 2. Diabetics had lower high-density lipoprotein (HDL) levels and greater total cholesterol, and triglycerides before and after training compared to controls. Training did not alter the plasma lipid profile for either group.

Euglycemic, Hyperinsulinemic Clamps and Insulin Sensitivity

Insulin infusion during the euglycemic clamp increased plasma insulin concentrations in the control subjects to 60 ± 3 μU/mL (average of 90- to 120-minute values) and 73 ± 8 μU/mL in the diabetic subjects ($P < .001$ *v* basal). Plasma insulin values tended to be higher throughout the clamp in the diabetic subjects. Training had no effect on insulin concentrations during the clamp in either group (Fig 2A). Plasma glucose concentrations for control subjects were maintained at euglycemia (between 90 and 100 mg/dL) with a variable glucose infusion throughout the clamps. Since fasting plasma glucose

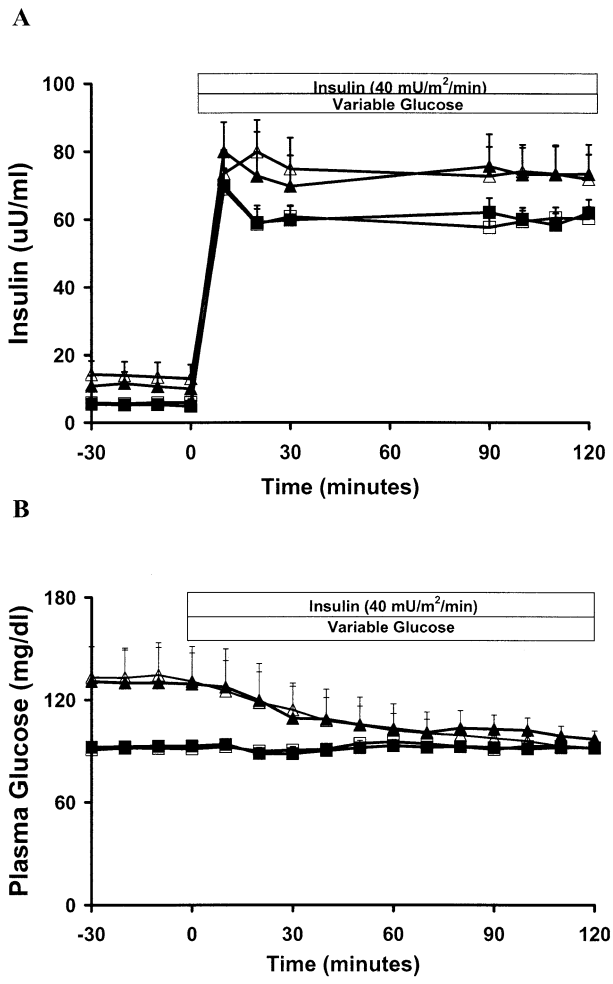

Fig 2. Plasma (A) insulin and (B) glucose concentrations during euglycemic, hyperinsulinemic clamps before and after training. Results shown are mean ± SEM for each time point. See text for statistical differences. Controls before training (■), controls after training (□), diabetics before training (▲), diabetics after training (△).

Table 3. Glucose Metabolism During Euglycemic, Hyperinsulinemic Clamps Before and After Training

	Controls				Diabetics			
	Before		After		Before		After	
Training	Basal	Insulin	Basal	Insulin	Basal	Insulin	Basal	Insulin
Disposal	2.8 ± 0.1	6.2 ± 0.5§	2.6 ± 0.1	7.1 ± 0.7†§	2.7 ± 0.2	4.3 ± 0.6‡	2.5 ± 0.4	5.5 ± 0.6†‡
Oxidation	2.1 ± 0.2	4.0 ± 0.3§	1.9 ± 0.2	4.0 ± 0.38§	1.2 ± 0.5*	1.8 ± 0.5*	0.7 ± 0.4*	1.8 ± 0.7*‡
Storage	0.7 ± 0.3	2.2 ± 0.3§	0.8 ± 0.2	3.1 ± 0.6†§	1.5 ± 0.7	2.5 ± 0.7‡	1.9 ± 0.7	3.7 ± 0.7*‡

NOTE. Data are given as means ± SEM in units of mg/kg FFM · min.
*$P < .05$ *v* controls.
†$P < .05$ *v* before training.
‡$P < .05$ *v* basal.
§$P < .001$ *v* basal.

concentrations were elevated in the diabetic subjects at the initiation of the insulin infusion (Table 1), their plasma glucose levels were allowed to fall until within the euglycemic range, and then maintained by a variable glucose infusion (Fig 2B).

Rates of glucose disposal were calculated using glucose specific activities. Rates of glucose and fat oxidation were calculated using indirect calorimetry to measure oxygen consumption (V_{O_2}) and CO_2 production (V_{CO_2}) continuously during the last 30 minutes of the basal state (time −30 to 0 minutes) and the last 30 minutes of insulin infusion (time 90 to 120 minutes). The rate of glucose storage was calculated as the difference between glucose disposal and oxidation. Before training, the rate of glucose disposal under basal conditions did not differ between the groups (Table 3). As expected, during insulin infusion, the diabetic subjects had significantly decreased glucose disposal (Table 3) compared to control subjects. Exercise training significantly increased insulin-stimulated (but not basal) glucose disposal in both groups, although the values in control subjects remained higher than those in the diabetic subjects ($P < .05$). In contrast, under basal conditions glucose oxidation rates were significantly lower in the diabetic subjects before and after training (Table 3). Insulin increased the rate of glucose oxidation in both groups ($P < .05$), but training did not affect basal or insulin-stimulated glucose oxidation. As a result, the increase in insulin-stimulated glucose disposal could be attributed to an increase in glucose storage. There was a strong correlation between insulin stimulated glucose disposal and insulin-stimulated glucose storage both before ($r = 0.70$) and after ($r = 0.87$) training (both $P < .001$). Moreover, the training-induced increments in glucose disposal and glucose storage were also correlated ($r = 0.79$, $P < .001$). The rate of glucose disposal was correlated with $V_{O_{2peak}}$ before ($r = 0.50$, $P < .05$) as well as after ($r = 0.55$, $P < .05$) training. Before training this correlation was due primarily to a correlation in the nondiabetics ($r = 0.50$, $P < .05$) that was absent in the diabetics ($r = 0.10$). Post-training, however, there was a correlation in nondiabetics ($r = 0.54$, $P < .05$) and diabetics ($r = 0.50$). However, the smaller sample size in the diabetics precluded the correlation from reaching statistical significance.

Fat oxidation rates are given in Table 4. Basal rates of fat oxidation were not significantly different between the 2 groups before and after training. While insulin infusion significantly decreased the rate of fat oxidation in control subjects ($P < .001$ *v* basal) before and after training, insulin infusion decreased fat oxidation in diabetic subjects only after training ($P < .05$ *v* basal). Nevertheless, insulin-stimulated rates of fat oxidation in diabetic subjects were significantly greater than control subjects both before and after training. Overall, training did not have an affect on fat oxidation in either group. The respiratory quotient, the ratio of V_{CO_2}/V_{O_2}, increased during insulin infusion in control subjects before and after training ($P < .001$ *v* basal), but was not significantly affected by insulin in diabetic subjects. Training did not have an effect in either group. These results reiterate that insulin stimulation increased glucose oxidation and inhibited fat oxidation in control subjects to a greater extent than in diabetic subjects and that, in both control and diabetic subjects, training did not affect the oxidative metabolism of glucose and fat (Tables 3 and 4).

Fasting endogenous glucose production (EGP) was not significantly different between the 2 groups before training (2.8 ±

Table 4. Fat Oxidation Rates and Respiratory Quotients During Euglycemic, Hyperinsulinemic Clamps

	Controls				Diabetic			
	Before		After		Before		After	
	Basal	Insulin	Basal	Insulin	Basal	Insulin	Basal	Insulin
Fat oxidation	1.05 ± 0.15	0.44 ± 0.10§	1.16 ± 0.14	0.46 ± 0.10§	1.22 ± 0.40	1.13 ± 0.30†	1.48 ± 0.22	1.00 ± 0.30*
RER	0.83 ± 0.01	0.92 ± 0.01§	0.82 ± 0.01	0.92 ± 0.01§	0.79 ± 0.04	0.81 ± 0.03†	0.76 ± 0.02†	0.82 ± 0.04†‡

NOTE. Data are given as means ± SEM in units of mg/kg FFM · min.
Abbreviations: RER, respiratory quotient.
*$P < .05$ *v* controls.
†$P < .01$ *v* controls.
‡$P < .05$ *v* basal.
§$P < .001$ *v* basal.

Table 5. IRS-1–Associated PI 3-Kinase Activity Before and After Training

	Controls		Diabetic	
	Before	After	Before	After
Basal	0.45 ± 0.06	0.36 ± 0.04*	0.55 ± 0.14	0.46 ± 0.12
Insulin	0.6 ± 0.11†	0.46 ± 0.06†	0.70 ± 0.15	0.56 ± 0.12
Delta	0.15 ± 0.08	0.10 ± 0.04	0.15 ± 0.08	0.10 ± 0.09

NOTE. Data are expressed as DPM relative to a rat liver standard and are given as mean ± SEM. PI 3-kinase activity was determined by measuring the incorporation of ATP32 into phosphatidylinositol by IRS-1 immunoprecipitates from muscle samples. See methods for details.

*$P < .05$ *v* before training.

†$P < .05$ *v* basal.

0.1 and 2.7 ± 0.3 mg/kg fat-free mass [FFM]/min, respectively). After training, fasting EGP was significantly lower in the diabetics compared to the controls (2.7 ± 0.1 and 2.2 ± 0.3 mg/kg FFM/min, respectively) ($P < .05$). In response to insulin infusion, EGP was completely suppressed by 10 minutes in the control subjects, and this suppression was not affected by training. In contrast, EGP was not completely suppressed until 30 minutes of insulin infusion in the diabetics both before and after training.

Insulin Signaling and GLUT4 Expression

IRS-1–associated PI 3-kinase activity, Akt serine phosphorylation, and protein expression as well as GLUT4 protein expression were determined in muscle lysates from muscle samples obtained basally and during the euglycemic, hyperinsulinemic clamps preformed before and after training. Before and after training muscle lysates for a given subject were always analyzed in the same activity assay or on the same immunoblot in order to reduce variability when evaluating the effect of training.

IRS-1–associated PI 3-kinase activities for control and diabetic subjects are given in Table 5. Insulin significantly increased IRS-1–associated PI 3-kinase activity in control but not in diabetic subjects ($P < .05$). Training decreased basal PI 3-kinase activity in control ($P < .05$) and diabetic subjects, although the decrease in the diabetic subjects did not reach statistical significance. Training had no effect on the ability of insulin to increase IRS-1–associated PI 3-kinase activity in either group.

Akt serine 473 phosphorylation and Akt protein expression before and after training were compared by immunoblot analysis. When expressed relative to protein content, Akt serine 473 phosphorylation was not significantly increased by training in either group (Fig 3A and B). However, examination of the immunoblots suggested that there was a training-induced increase in Akt protein expression in both groups. Muscle lysates from the groups were matched and analyzed on the same blot to reduce intragroup and intergroup variability. The results in Fig 4 show that before training, Akt protein expression for diabetic subjects was reduced by 29% ± 3% compared to control subjects ($P < .001$). In response to training, Akt protein expression increased 19% ± 5% for control subjects ($P < .001$ *v* before training) and 30% ± 10% for diabetic subjects ($P < .001$ *v* before training). However, Akt protein expression after training in diabetics was still significantly decreased compared to controls after training ($P < .001$), although it did not differ from levels seen in controls before training.

Likewise, comparison of GLUT4 protein expression was performed by analyzing basal muscle lysates before and after training on the same immunoblot to reduce variability between the groups. This analysis showed that before training, GLUT4 protein expression was 45 ± 5 and 57 ± 7 arbitrary units in control and diabetic subjects, respectively (P = not significant [NS]) Training increased GLUT4 expression by 38% ± 8% in the control subjects ($P < .001$) and by 22% ± 10% in the diabetic subjects ($P < .05$). There was no correlation between the increase in GLUT4 protein expression and insulin-stimulated glucose disposal in response to training.

Glycogen Synthase Activity

Before training, basal values for $GS_{0.1}$ and GS_{10} (total) were not different between control and diabetic subjects. Likewise, insulin increased $GS_{0.1}$ to a similar extent in both groups (Table 6). Exercise training significantly increased GS_{10} activity in both groups, with concomitant increases in $GS_{0.1}$, such that GS_{FV} was not altered.

The results from both groups were combined in order to investigate the relationships between insulin's ability to stimulate $GS_{FV,}$ glucose disposal, and glucose storage before and after training. Before training, insulin-stimulated GS_{FV} was not correlated with insulin-stimulated glucose storage ($r = 0.15$, P = NS). However, after training, insulin-stimulated GS_{FV} was significantly correlated with insulin-stimulated glucose storage ($r = 0.53$, $P < .05$) and tended to correlate with insulin-stimulated glucose disposal ($r = 0.41$, $P = .06$).

DISCUSSION

Insulin resistance characterizes obese and type 2 diabetic subjects.[34-36] Although exercise training is widely believed to enhance insulin sensitivity in such subjects,[28,37,38] the mechanisms responsible for this effect are unclear. The present study was undertaken to assess the relative contributions of improvements in insulin receptor signaling, GLUT4 protein expression, and glycogen synthase activity to the training-induced increase in insulin sensitivity. To accomplish this purpose, we employed a progressively increasing training program for 8 weeks in overweight, sedentary nondiabetic control subjects and patients with type 2 diabetes. Aerobic capacity is positively associated with insulin sensitivity,[39-41] suggesting a link between physical activity and insulin sensitivity. In the present study, 8 weeks of aerobic exercise training in insulin-resistant type 2 diabetic and matched nondiabetic control subjects led to significant improvements in aerobic capacity (Vo_{2peak}) and insulin sensitivity (glucose disposal and storage). There was a positive correlation between Vo_{2peak} and insulin-stimulated glucose disposal before and after training. Vo_{2peak} before training was significantly lower in the diabetic subjects compared to the controls, consistent with previous studies[42,43] and this initially lower Vo_{2peak} may have contributed to the significantly greater percentage increase in Vo_{2peak} that was observed in the diabetic subjects as a result of training.[42]

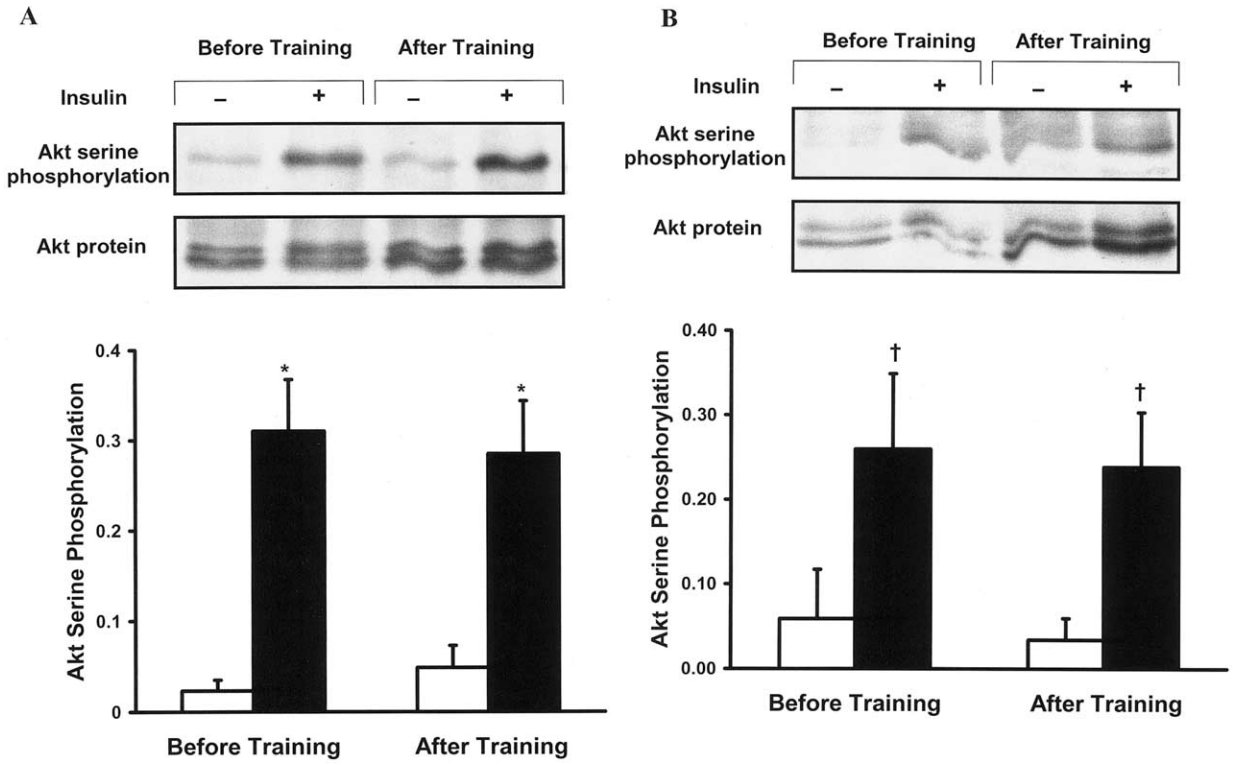

Fig 3. Effect of training on Akt serine phosphorylation for (A) control and (B) diabetic subjects. Subjects underwent a euglycemic, hyperinsulinemic clamp (40 mU/m^2/min) with biopsies of the vastus lateralis muscle basally and after 30 minutes of insulin infusion. Representative immunoblots of Akt serine 473 phosphorylation and Akt protein expression are shown on the top of the Figs. Muscle lysates were resolved by SDS-polyacrylamide gel electropheresis (SDS-PAGE), and proteins were transferred to nitrocellulose membranes, which were probed with anti-Akt serine 473 phosphorylation antibodies and detected with enhanced chemiluminescence (ECL). Membranes were stripped and reprobed with an anti-Akt antibody to determine protein expression. Quantification was accomplished by scanning densitometry. Akt serine 473 phosphorylation relative to protein expression are presented as mean ± SEM for basal (□) and insulin-stimulated (■) conditions. *$P <$ .001 *v* basal, †$P <$ .05 *v* basal.

The present study was undertaken, in part, to determine whether training-induced improvements in insulin sensitivity could be attributed to increased insulin signaling. Insulin signaling is reduced in obese nondiabetic subjects compared to lean controls and decreased to an even greater extent in patients with type 2 diabetes mellitus.[12,13,44] In an earlier study, a single bout of exercise increased insulin receptor and IRS-1 tyrosine phosphorylation in insulin-resistant subjects without increasing glucose disposal.[12] Therefore, we focused on the downstream signaling elements, IRS-1–associated PI 3-kinase activity and Akt serine 473 phosphorylation. Consistent with previous results in obese and diabetic subjects,[12,13] insulin minimally stimulated IRS-1–associated PI 3-kinase activity in both groups before training. After 8 weeks of exercise training, even though insulin-stimulated glucose disposal was improved in both groups, insulin stimulation of IRS-1–associated PI 3-kinase activity was unchanged. This is similar to the situation in the obese Zucker rat model of insulin resistance, in which 7 weeks of exercise training improved insulin sensitivity without any effect on insulin signaling at the level of PI 3-kinase.[14] In another study, 7 days of training had no effect on PI 3-kinase activity in insulin-resistant obese subjects.[28] In contrast, Houmard et al demonstrated that insulin activation of phosphotyrosine associated PI 3-kinase was enhanced as a result of 7 days of exercise training in young healthy individuals.[45] Therefore, although improvements in insulin signaling may contribute to short-term training-enhanced insulin sensitivity in lean, healthy controls, this does not appear to be the case in overweight, sedentary subjects who underwent a longer period of exercise training. In such subjects, other mechanisms are likely to be responsible for the improvement in insulin action. Nevertheless, it should be cautioned that a small increase in insulin stimulation of PI 3-kinase, undetectable be the methods used here, may have been present and contributed to the effects of training.

In contrast to PI 3-kinase, Akt serine 473 phosphorylation was dramatically increased in response to insulin infusion.[16] In the present study, Akt expression in the diabetic subjects before training was significantly decreased compared to the obese nondiabetic subjects. There have been contradictory reports on the level of Akt expression in insulin-resistant versus insulin-sensitive subjects.[16,19,46] Our results suggest that there may be a gradation of Akt expression within insulin resistance, with diabetes conferring an additional decrease. Regardless, exercise training increased Akt expression significantly in each group.

The present study was also designed to assess the relative

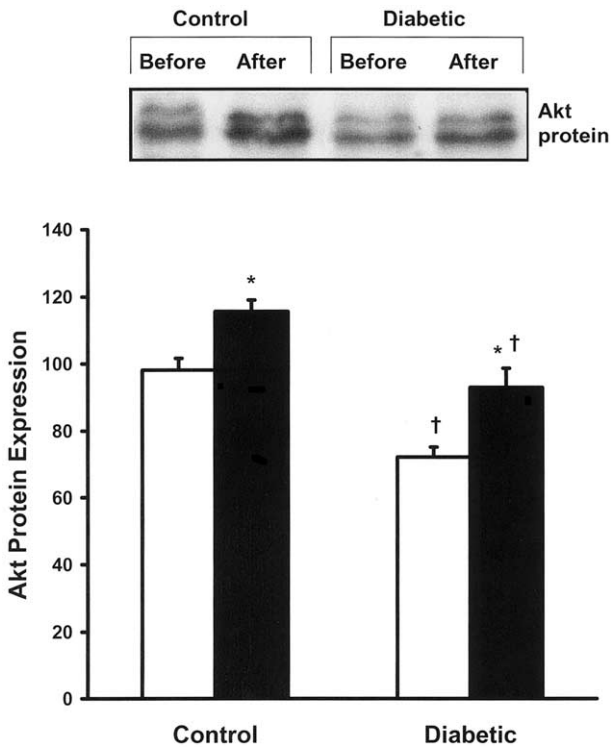

Fig 4. Effect of training on Akt protein expression in control and diabetic subjects. Akt protein expression was determined in basal samples of muscle lysates using immunoblot analysis. Before and after training muscle samples for each subject were run on the same immunoblot to decrease variability in the effect of training. Furthermore, muscle lysates from control and diabetic subjects were run on the same immunoblot to decrease inter-group variability. A typical immunoblot is shown. Quantification of the results by scanning densitometry are shown for both groups before (□) and after (■) training. Data are presented as mean ± SEM. *$P < .001$ *v* before training, †$P < .001$ *v* control subjects.

roles of downstream effectors of insulin action on training-induced insulin sensitization. Unlike the effects of training on insulin signaling, a training-induced increase in GLUT4 expression seems to be common to all populations studied,[20,25] and was evident in the current study with GLUT4 expression increasing an average of 38% in nondiabetic and 22% in diabetic subjects. It has also been suggested that the training-induced increase in GLUT4 expression can compensate for the signaling defects seen in the obese Zucker rat[47] and that the addition of insulin-sensitive glucose transporters in skeletal muscle can be sufficient to enhance whole body glucose disposal in response to training. The mechanism by which an increase in GLUT4 expression improves muscle insulin sensitivity is unclear, particularly when insulin resistance is thought to result from a defect in GLUT4 translocation rather than decreased GLUT4 protein. Brozinick et al observed that there was an increase in GLUT4 associated with the plasma membrane fraction, but not with the microsomal membrane fraction after exercise training in obese Zucker rats.[48] Furthermore, under basal conditions cell surface GLUT4 was not enhanced, but with insulin stimulation cell surface GLUT4 (determined by photoaffinity labeling of exo-facial transporters) was significantly increased.[49] These results suggest that training induced changes in GLUT4 expression may set the stage for immediate insertion into the plasma membrane in response to insulin stimulation, thereby in some way bypassing GLUT4 translocation. The results of the present study support this hypothesis in that training did not amend the defects in the insulin signaling pathway proposed to control GLUT4 translocation, but increased GLUT4 protein expression. However, in the present study there was no correlation between the training-induced increase in GLUT4 expression and insulin stimulated glucose uptake. This finding confirms earlier reports,[20] and suggests that the increase in GLUT4 expression may be more relevant to the response of specific pools of GLUT4 transporters to exercise. Regardless of the specific mechanism involved, a training-induced increase in GLUT4 protein expression has been consistently reported and is likely that increased GLUT4 expression plays at least a partial role in increased skeletal muscle insulin sensitivity after training.

In the present study, the training-induced improvement in insulin-stimulated glucose disposal was strongly associated with improved insulin-stimulated glycogen storage. Impaired insulin-stimulated glycogen synthase activity and systemic glucose storage (muscle glycogen synthesis) are consistent findings in insulin resistance.[8,50] Consistent with this, there was minimal glycogen synthase activation in response to insulin before training in both overweight nondiabetic and obese type 2 diabetic subjects. In response to training, both absolute $GS_{0.1}$

Table 6. Glycogen Synthase Activity Before and After Training

		Before Training		After Training	
		Basal	Insulin	Basal	Insulin
Controls	$GS_{0.1}$	1.35 ± 0.30	1.80 ± 0.35‡	2.10 ± 0.40†	2.67 ± 0.33†
	GS_{10}	12.52 ± 1.44	11.15 ± 1.52	17.06 ± 3.36†	15.13 ± 1.49*
	GS_{FV}	0.10 ± 0.01	0.15 ± 0.02§	0.12 ± 0.02	0.18 ± 0.02§
Diabetics	$GS_{0.1}$	1.85 ± 0.63	2.49 ± 0.57‡	2.59 ± 0.90*	2.77 ± 0.70
	GS_{10}	14.43 ± 2.50	13.59 ± 1.81	20.84 ± 2.49†	18.04 ± 2.44†
	GS_{FV}	0.11 ± 0.02	0.18 ± 0.03§	0.11 ± 0.03	0.14 ± 0.03

NOTE. Glycogen synthase activity was assayed in the presence of 0.1 ($GS_{0.1}$) and 10 (GS_{10}) mmol/L glucose 6-phosphate. Glycogen fractional velocity (GS_{FV}) is the ratio of $GS_{0.1}/GS_{10}$. Data are presented as means ± SEM.

*$P < .05$ and †$P < .01$ *v* before training.

‡$P < .05$ and §$P < .01$ *v* basal.

activity and glycogen storage were significantly improved in the nondiabetic and diabetic subjects, with insulin-stimulated $GS_{0.1}$ activity being significantly greater than before training. These findings are consistent with other reports of increased glycogen synthase activity in response to training.[23,51] While insulin-stimulated glycogen synthase activity was not significantly affected by training in the diabetic subjects, insulin-stimulated $GS_{0.1}$ after training tended to be increased compared to before training and the increase in glucose disposal could be accounted for by the difference in glucose storage after training. The increases in $GS_{0.1}$ are consistent with the increased total activity of glycogen synthase in both control and diabetic subjects, which is likely to reflect an increase in glycogen synthase protein. Such an increase suggests that like GLUT4, an increase in glycogen synthase expression is associated with training-induced improvements in insulin sensitivity.

The present findings regarding the effects of training may be placed into perspective by comparison to normative data from a group of lean (BMI = 25.0 ± 0.2), healthy control subjects of similar age and ethnic composition whose data we have previously reported.[12] Using the same insulin infusion rate, the lean subjects studied without training had an insulin-stimulated glucose disposal rate of approximately 6.5 mg/(kg FFM/ min), so training increased glucose disposal in the overweight, sedentary nondiabetic subjects in the present study to normal levels. The diabetics in the present study, after training, approached values seen in the lean controls from the earlier study. Insulin stimulation of PI 3-kinase activity doubled in lean controls studied without training, but after training still did not increase in the present study in either group, accentuating the findings that other mechanisms are responsible for the training effect. This is consistent with our findings in the Zucker Fatty rat.[14] Glycogen synthase activities increased by 40% to 50% in response to training in the current study, and in our previous report, obese and diabetic subjects had a 25% to 40% decrease in glycogen synthase activity compared to the controls. Therefore, like glucose disposal, glycogen synthase activities approached normal after training.

The results of the present study show that overweight, sedentary nondiabetic and type 2 diabetic subjects responded metabolically in much the same manner to exercise training. Both groups had similar improvements in Vo_{2peak} and insulin-stimulated glucose disposal. Both groups had increased total glycogen synthase activity and GLUT4 and Akt protein expression; neither group had improved insulin receptor signaling through PI 3-kinase. The lack of response of insulin receptor signaling was similar to the lack of improvement observed in insulin-resistant subjects trained for 7 days,[28] but different from the finding that healthy insulin-sensitive subjects responded to seven days of exercise training with improved insulin receptor signaling through PI 3-kinase.[45] It should be pointed out that in the present study, even though the average age of the diabetic subjects was slightly greater than the obese nondiabetic subjects, their responses to training were similar. Regardless, the age difference, as well as ethnic composition of the groups, may have affected the results. However, since both groups responded (or did not respond) similarly, the effects of the age and ethnicity discrepancies may have been relatively minor.

In summary, the present study assessed the relative contributions of improvements in the PI 3-kinse pathway of insulin receptor signaling, GLUT4 expression, and glycogen synthase activity to the exercise-induced increase in insulin sensitivity. The following working model for training-induced improvements in insulin action in skeletal muscle from insulin resistant subjects can therefore be proposed. An increase in GLUT4 protein expression combined with an increase in Akt expression may result in a proportional increase in GLUT4 appearance at the plasma membrane and allow a greater flux of glucose into the cell. Once inside the myocyte, glucose then may be shunted preferentially to glycogen via the increased activity/expression of glycogen synthase. It is always possible that different training regimens or exercise accompanied by weight loss (which did not occur in the present study) could bring about different adaptive mechanisms.

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